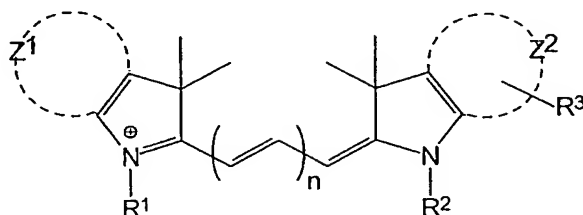


Claims

1. A matched set of fluorescent dyes comprising at least two different fluorescent dyes of formula (I):

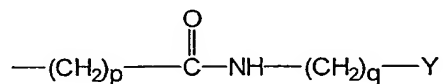


(I)

wherein  $n$  is different for each said dye and is 1, 2, or 3;

$Z^1$  and  $Z^2$  independently represent the carbon atoms necessary to complete a phenyl or naphthyl ring system;

one of groups  $R^1$  and  $R^2$  is the group:



where  $Y$  is a target bonding group;

remaining group  $R^1$  or  $R^2$  is selected from  $-(CH_2)_4-W$  or  $-(CH_2)_r-H$ ;

group  $R^3$  is hydrogen, except when either  $R^1$  or  $R^2$  is  $-(CH_2)_r-H$ , in which case  $R^3$  is  $W$ ;

$W$  is selected from sulphonic acid and sulphonate;

$p$  is an integer from 3 to 6;

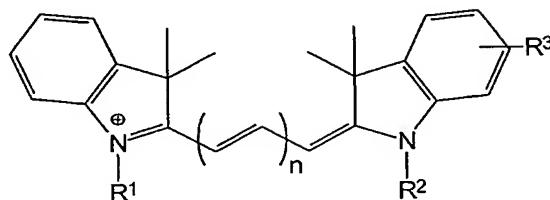
$q$  is selected to be 2 or 3; and

$r$  is an integer from 1 to 5;

and their salts;

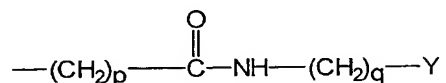
characterised in that when  $n$  of two of said dyes differs by + 1, one of  $p$ ,  $q$  and  $r$  of said two dyes differs by -1.

2. A matched set of fluorescent dyes comprising at least two different fluorescent dyes of formula (II):



(II)

wherein  $n$  is different for each said dye and is 1, 2, or 3;  
one of groups  $R^1$  and  $R^2$  is the group:



15

where  $Y$  is a target bonding group;

remaining group  $R^1$  or  $R^2$  is selected from  $-(CH_2)_4-W$  or  $-(CH_2)_r-H$ ;

group  $R^3$  is hydrogen, except when either  $R^1$  or  $R^2$  is  $-(CH_2)_r-H$ , in which case  $R^3$  is  $W$ ;

20  $W$  is selected from sulphonic acid and sulphonate;

$p$  is an integer from 3 to 6;

$q$  is selected to be 2 or 3; and

$r$  is an integer from 1 to 5;

and their salts;

25 characterised in that when  $n$  of two of said dyes differs by + 1, one of  $p$ ,  $q$  and  $r$  of said two dyes differs by -1.

3. A matched set according to claim 1 or claim 2 comprising at least two different fluorescent dyes according to formula (I) or (II) in which:

30  $n$  is selected to be 1 or 2;

$p$  is selected to be 4 or 5;

q is selected to be 2 or 3; and

r is selected to be 1, 2 or 3.

4. A matched set according to any of claims 1 to 3 wherein said  
5 target bonding group Y in each dye of the set of dyes is the same and is  
selected from a maleimido group and an iodoacetamido group.

5. A matched set according to claim 4 wherein in each said dye Y is a  
maleimido group.

10

6. A matched set according to any of claims 1 to 5 wherein said salts  
are selected from  $K^+$ ,  $Na^+$ ,  $NH_4^+$ ,  $R_3NH^+$  and  $R_4N^+$  where R is  $C_1$  to  $C_4$   
alkyl.

15 7. A matched set of dyes according to any of claims 1 to 6 selected  
from:

#### Set 1

20 1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-2-  
[(1*E*,3*E*)-3-(1-ethyl-3,3-dimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-  
ylidene)prop-1-enyl]-3,3-dimethyl-3*H*-indolium (Compound I); and  
1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-  
3,3-dimethyl-2-[(1*E*,3*E*,5*E*)-5-(1,3,3-trimethyl-5-sulpho-1,3-dihydro-2*H*-  
25 indol-2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound II);

#### Set 2

1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-2-  
30 [(1*E*,3*E*)-3-(1-propyl-3,3-dimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-  
ylidene)prop-1-enyl]-3,3-dimethyl-3*H*-indolium (Compound III); and

1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-3,3-dimethyl-2-[(1*E*,3*E*,5*E*)-5-(1-ethyl-3,3-trimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound IV);

5    Set 3

1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-2-[(1*E*,3*E*)-3-(1-ethyl-3,3-dimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-ylidene)prop-1-enyl]-3,3-dimethyl-3*H*-indolium (Compound I); and

10  1-(5-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxopentyl)-3,3-dimethyl-2-[(1*E*,3*E*,5*E*)-5-(1-ethyl-3,3-trimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound V);

Set 4

15

1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-2-[(1*E*,3*E*)-3-(3,3-dimethyl(1-sulpho-butyl)-1,3-dihydro-2*H*-indol-2-ylidene)prop-1-enyl]-3,3-dimethyl-3*H*-indolium (Compound VI); and

20  1-(5-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxopentyl)-3,3-dimethyl-2-[(1*E*,3*E*,5*E*)-5-(3,3-dimethyl-(1-sulpho-butyl)-1,3-dihydro-2*H*-indol-2-ylidenè)penta-1,3-dienyl]-3*H*-indolium (Compound VII).

Set 5

25  1-(6-{[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]amino}-6-oxohexyl)-2-[(1*E*,3*E*)-3-(1-ethyl-3,3-dimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-ylidene)prop-1-enyl]-3,3-dimethyl-3*H*-indolium (Compound VIII); and

30  1-(6-{[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino}-6-oxohexyl)-3,3-dimethyl-2-[(1*E*,3*E*,5*E*)-5-(1-ethyl-3,3-trimethyl-5-sulpho-1,3-dihydro-2*H*-indol-2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound IV); and

Set 6

1-(6-[[3-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)propyl]amino]-6-oxohexyl)-2-  
[(1*E*,3*E*)-3-(3,3-dimethyl(1-sulpho-butyl)-1,3-dihydro-2*H*-indol-2-ylidene)prop-  
5 1-enyl]-3,3-dimethyl-3*H*-indolium (Compound IX); and  
1-(6-[[2-(2,5-dioxo-2,5-dihydro-1*H*-pyrrol-1-yl)ethyl]amino]-6-oxohexyl)-3,3-  
dimethyl-2-[(1*E*,3*E*,5*E*)-5-(3,3-dimethyl-(1-sulpho-butyl)-1,3-dihydro-2*H*-indol-  
2-ylidene)penta-1,3-dienyl]-3*H*-indolium (Compound X).

10 8. A method for labelling a mixture of proteins in a sample wherein each  
of said proteins contains one or more cysteine residues, said method  
comprising:

- i) adding to an aqueous liquid containing said sample a fluorescent dye  
selected from a matched set of fluorescent dyes wherein each said dye  
15 contains a target bonding group that is covalently reactive with said proteins;  
and  
ii) reacting said dye with said proteins so that said dye labels said  
proteins;  
characterised in that all available cysteine residues in said proteins are  
20 labelled with said dye.

9. A method according to claim 8 wherein said fluorescent dye is a  
cyanine dye.

25 10. A method according to claim 9 wherein said cyanine dye contains a  
sulphonic acid or sulphonate group.

11. A method according to any of claims 8 to 10 wherein said target  
bonding group is selected from a maleimido group and an iodoacetamido  
30 group.

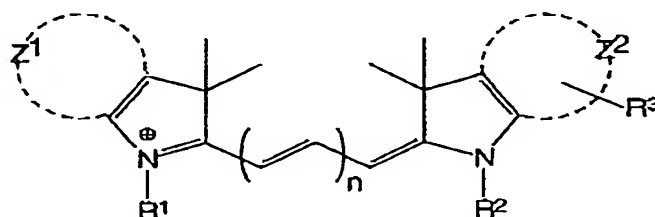
12. A method according to claim 8 further comprising prior to step i), the  
step of treating the protein with a reductant.

13. A method according to claim 8 wherein said dye is used in a range of 5 to 200nmol of dye per 50µg of protein.

14. A method according to claim 8 wherein said labelling is performed at a pH in the range from 6.0 to 9.0.

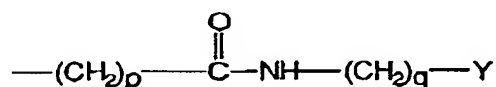
15. A method for labelling one or more proteins in a sample, the method comprising:

i) adding to a liquid sample containing said one or more proteins a fluorescent dye selected from a matched set of fluorescent dyes each dye in said set having the formula (I):



(I)

wherein n is different for each said dye and is 1, 2, or 3;  
Z<sup>1</sup> and Z<sup>2</sup> independently represent the carbon atoms necessary to complete a phenyl or naphthyl ring system;  
one of groups R<sup>1</sup> and R<sup>2</sup> is the group:



where Y is a target bonding group;  
remaining group R<sup>1</sup> or R<sup>2</sup> is selected from -(CH<sub>2</sub>)<sub>4</sub>-W or -(CH<sub>2</sub>)<sub>r</sub>-H;  
group R<sup>3</sup> is hydrogen, except when either R<sup>1</sup> or R<sup>2</sup> is -(CH<sub>2</sub>)<sub>r</sub>-H, in which case R<sup>3</sup> is W;  
W is selected from sulphonic acid and sulphonate;  
p is an integer from 3 to 6;  
q is selected to be 2 or 3; and

r is an integer from 1 to 5;

and their salts;

characterised in that when n of two of said dyes differs by +1, one of p, q and r of said two dyes differs by -1; and

- 5 ii) incubating said dye with said sample under conditions suitable for labelling said one or more proteins.

16. A method according to claim 15 wherein each of  $Z^1$  and  $Z^2$  represents the carbon atoms necessary to complete a phenyl ring system.

10

17. A method according to claim 15 or claim 16 wherein:

n is selected to be 1 or 2;

p is selected to be 4 or 5;

q is selected to be 2 or 3; and

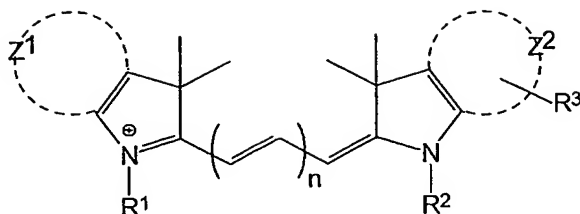
15 r is selected to be 1, 2 or 3.

18. A method according to any of claims 15 to 17 wherein said target bonding group Y is selected from a maleimido group and an iodoacetamido group.

20

19. A kit comprising a matched set of fluorescent dyes comprising at least two different fluorescent dyes having the formula (I):

25

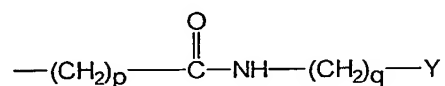


(I)

wherein  $n$  is different for each said dye and is 1, 2, or 3;

$Z^1$  and  $Z^2$  independently represent the carbon atoms necessary to complete a phenyl or naphthyl ring system;

one of groups  $R^1$  and  $R^2$  is the group:



where  $Y$  is a target bonding group;

remaining group  $R^1$  or  $R^2$  is selected from  $-(CH_2)_4-W$  or  $-(CH_2)_r-H$ ;

group  $R^3$  is hydrogen, except when either  $R^1$  or  $R^2$  is  $-(CH_2)_r-H$ , in which case  $R^3$  is  $W$ ;

$W$  is selected from sulphonic acid and sulphonate;

$p$  is an integer from 3 to 6;

$q$  is selected to be 2 or 3; and

$r$  is an integer from 1 to 5;

and their salts;

characterised in that when  $n$  of two of said dyes differs by + 1, one of  $p$ ,

$q$  and  $r$  of said two dyes differs by -1.